Chemical Studies on the Freshness of Whale Meat. I.

Evaluation of freshness and changes in quantity of several kinds of nitrogen in whale meat following deterioration of freshness.

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There is generally a lapse of time on the sea between the time when a whale is harpooned and until it is dissected. There is still a longer lapse of time before this prepared whale meat gets into the hand of consumers. It follows, therefore, that there should be more chance of deterioration of freshness in whale meat than in the meat of domestic animals and fowls. Preservation method and use of whale meat varies according to the degree of freshness. It can be said, therefore, that the question of the freshness of whale meat has the most important significance in the handling of whale meat.

The author has undertaken the present studies with the object of making chemical investigations on the deterioration of freshness of whale meat and of making a contribution to the problem of maintaining freshness.

First, it is necessary to decide on the evaluation method of the freshness of whale meat in the author's future experiments.

An example of the evaluation method used by operators in the field in Japan is shown in Table I.[1], but it is too subjective to be adopted as the method in the author's experiments, and even in the field it could only be used at the time of dissection.

Table I. Standard of Freshness of Whale Carcass.

Grade	Colour and lustre of flesh	Elasticity of muscle.	Offensive smell	Exudation of blood	Lapse of time between killing and dissection
Very fresh (90%)	Very dark red, semitransparent, lustrous	Conspicuous	none	none	Less than 6 hrs.
Fresh (80%)	More reddish, lustrous	Recognizable	〃	Recognizable by pressing	〃 12 〃
Satisfactory (60%)	Reddish grey, non-lustrous	none	〃	Recognizable	〃 18 〃
Unsatisfactory (40%)	〃	〃	Recognizable	Conspicuous	〃 24 〃
Poor (Less than 40%)	〃	〃	Extreme	Flowing	More than 25 hrs.

Chemical methods for determination of the quality of flesh have been worked out by many researchers already and among them, the method based

on the determination of various kinds of nitrogen has been studied most and it seems to be more reliable than the others, as for instance, oxygen consumption test, methylene blue and nitrate redution test and hydrogen sulfide test.

According to Malin[2], high-quality meat, fish and fowl shows 6—16 mg, satisfactory fish and fowl, 18—26 mg and poor quality meat, fish and fowl, 27 or more mg total NH_3 per 100 g.

Tillmans and Otto[3] reported that at the beginning of the decomposition of fish, NH_3 reached 30 mg and amino acid N 100 mg per 100 g, and Shimizu[4] found ammonia N 30—40 mg and amino acid N 80 mg per 100 g at the time.

Glassmann and Rochwarger[5] reported that 0.020% and 0.025% are the respective critical quantity of NH_3 indicating the beginning of putrefaction in the flesh of warm-blooded animals and of fish.

Herzner and Mann[6] observed that the amount of pure albumin in meat, which decreases with the progress of putrefaction, gave an indication of incipient putrefaction and that a pH of over 6.2 indicated putrefaction in meat.

Ipatov[7] recommended the determination of hydrolized protein for evaluation of the freshness of meat.

For measurement of spoilage in fish, Beatty and Gibbons[8] recommended the determination of trimethyl amine fraction in the filtered muscle juice. They found that fresh muscle showed a mean value of 0.17 mg of trimethyl amine N in each 100 cc of muscle juice; that odors appeared at about 4.0—6.0 mg and that the trimethyl amine fraction did not increase appreciably by autolysis.

For detecting incipient decay in meat, Brotzu[9] recommended the determinations of amino acid N and pH of meat juice which transuded from minced meat by heating, and of the bacterial count of juice obtained by pressing the minced meat. He found that 300—350 mg of amino acid N (Sörensen) per 100 cc of meat juice and pH 6.3 and above indicated incipient decomposition.

According to Yamamura[10] there is a straight linear relation between ammonia content and pH of fish. He observed that ammonia content at the beginning of putrefaction was 30 mg per 100 g, as was found by Tillmans and Otto, and the colorimetric measurement of pH at this period

was 6.5.

The present paper embodies the author's observations on the changes in the amount of some nitrogen in whale meat during the course of deterioration of freshness, which have been carried out with the object of determining of nitrogen which would indicate the degree of freshness in whale meat.

I. Changes in the amount of volatile basic N, non-protein N, formol titrating N, trimethyl amine N and trimethyl amine-oxide N were determined in minced whale meat standing at room temperature.

The material used in the experiment was the 'oniku', a portion of flesh between the dorsal fin and tail, of blue whale (shironagasu-kujira; *Balaenoptera musclus*). Its fat content was very high, namely 29.2%. Samples, taken from the minced material, showed a distinct acid reaction and emitted strong sour odor all through the course of experiment. The author believes that this was due to the formation of a remarkable amount of free low fatty acids resulting from the decomposition of fat by enzyme in muscles and that, consequently, the propagation and the kind of bacteria was considerably restricted.

The amounts of volatile basic N and formol titrating N increased regularly with lapse of time, and the rate of increase was great (Table III and V). The increare of non-protein N (Table IV) and trimethyl amine N (Table V) were comparatively small. Change in the amount of trimethyl amine-oxide was practically unnoticed (Table V).

On account of the strong sour odor, it was difficult to detect the putrid odor at the incipient stage of putrefaction of protein in the sample. However, assuming that the onset of putrefaction took place when gas generation in the interior of a sample (formation of bubbles) was detected, it was found that the amount at this period were: volatile basic N, 30.2 and 29.7 mg per 100 g, non-protein N, 500 mg, formol titrating N, 136 mg and trimethyl amine N, 0.45 mg.

II. Comparison was then made of the percentage value of freshness which, as shown in Table I, is determined rather subjectively by the whale-fishing men in Japan for evaluating the degree of freshness at the time of dissection, and the quantities of volatile basic nitrogen and of formol titrating nitrogen.

The material used in this experiment was flesh of sperm whale (mak-

kō-kujira ; *Physeter macrocephalus*).

The results obtained are listed in Table VI and also summarized in Table II.

Table II

Freshness % (cf. Table I)	Volatile basic N (mg/100 g)	Formol titrating N (mg/100 g)	Number of samples used
90	3.5	61.5	1
85	5.5	70.7	4
80	8.2	81.2	5
75	11.5	90.2	4
70	16.0	95.8	1
60	38.6		1
50	246		1
40	453		1

With the progress of deterioration from 90% to 70%, the mean value of nitrogen for each percentage value shows gradual and yet a definite increase, as shown in Table II. However, there are some inconsistencies among the individual measurement values for different percentage values, as shown in Table VI.

During the course of deterioration under 70%, quuntitiy of volatile basic N shows a sudden increase. Formol titrating N during this period was not determined owing to the matter of sampling.

These results indicate that the change in the percentage values which are determined by experts, corresponds to that in the amount of volatile basic and formol titrating nitrogen.

From the results of these experiments, it has become clear that the freshness of whale meat can be evaluated by determining the volatile basic nitrogen or formol titrating nitrogen.

In author's future experiments, therefore, comparison on the degree of freshness of whale meat will be done by the comparison on the amount of volatile basic nitrogen or of formol titrating nitrogen.

Experimental

I. Deterioration of freshness of minced whale meat at room temperature.

a) Change in the quantity of volatile basic nitrogen.

Material and treatment:— The material used was the refrigerated 'oniku,' the portion of flesh between the dorsal fin and tail, of blue whale (shironagasu-kujira, *Balaenoptera musclus*) caught in the Antarctic Occan.

Analytical data of the material are shown in the following table.

Moisture	Crude protein	Crude fat	Ash
54.70%	15.58%	29.20%	0.64%

The minced, pounded and thoroughly mixed material was devided into many Petri' dishes. All the dishes were put in an air-tight glass vessel containing a small amount of water in the bottom and kept at room temprature. After standing for a definite time, a dish was taken out from the vessel for a determination, one dish for each value. The amount of a in sample in one dish was about 50 g. Temperature at noon during the course of experiments, 20°--22°C.

Method of determination:— The determination was made by the aeration method.[11),12)] 4 g of thoroughly mixed sample was introduced into a test-tube, and 20 cc of distilled water, 0.5 g MgO, 0.1 g NaF, and a few drops of octyl alcohol to prevent foaming, were added. By means of a fairly rapid flow of air current throtgh the mixture at 40°C by suction, volatile basic substance was driven out into 10—15 cc of 0.02 *N* H_2SO_4. After 3 hrs' continued aeration, excess acid was titrated with 0.02 *N* NaOH with methyl red-methylene blue indicator.

Results obtained are shown in Table III.

Table III.

Time (hrs)	Volatile basic N (mg/100 g)	Indications	Time (hrs)	Volatile basic N (mg/100 g)	Indications
0	13.2		165	35.7	Distinct gas evolution and putrid smell.
21	17.3	Faint sour odor.	189	38.4	
45	20.3	Strong sour odor.	213	40.5	
69	23.6		262	47.1	
93	27.2	Sour odur, slightly diminished Gas generation in the interior.	310	50.3	
117	30.2		381	53.7	
141	33.0				

b) Change in the amount of non-protein nitrogen.

Material and treatment:— Same as in a). Temperature at noon during the experiment, 20°—22°C.

Method of determination:— 5 g of thoroughly mixed sample was weighed into a mortar, thoroughly pounded with 0.5 g of emery powder, transferred to a beaker, washed out with 50 cc of distilled water and 5 cc of ether and heated on a steam bath for 10 min. under occasional stirring.

25 cc each of Barnstain's $CuSO_4$ solution and NaOH solution were added and after standing for 30 min., supernatant liquid was decanted on filter-paper, collecting the filtrate in 500 cc volumetric flask. The precipitate was washed with 50 cc distilled water, and, after standing for 15 min., the liquid was decated as before. After the washings were repeated using six 50 cc portions of water, the combined filtrate and washings were diluted with distilled water to the volume indicated and mixed thoroughly. 10 cc of this solution was used for the determination of nitrogen by the Kjeldahl method.

Results obtained are listed in Table IV.

Table IV.

Time (hrs)	Non-protein N (mg/100 g)	Indications	Time (her)	Non-protein N (mg/100 g)
0	460		116	539
20	469	Faint sour odor.	140	562
44	478	Distinct sour odor.	190	611
68	497		237	642
92	500	Sour odor and faint putrid smell.		

c) Changes in the amount of formol titrating N, volatile basic N, trimethyl amine N, and trimethyl amine-oxide N. Material and treatment:— Material used was the same as in a) and b), but somewhat inferior in freshness. Treated as above. Temperature at noon during the experiment, 29°—30°C.

Method of determinaton :—

Formol titrating N— 100 g of throughly mixed sample was weighed into a beaker, 300 cc of distilled water added, and heated in a water bath for 20 min., under stirring. After standing for a short time, the liquid was decanted into an Erlemeyer's flask, and liquid was squeezed out of the meat residue in the beaker with a piece of gauze. The residue was extracted three times with successive 200 cc portions of distilled water as in the foregoing. To the combined turbic liquid in the flask was added 10 cc of 20% sulfosalicylic acid, and after a few hours, the mixture was filtered through a moistened filter paper, collecting the filtrate in 1 *l* volumetric flask. The residue on the filter was washed with 0.2% sulfosalicylic acid solution until the combined filtrate and washings reach the marking on the flask. The solution thus obtained is hereafter called solution [A]. 20 cc of the solution [A] was submitted to formol titration (A. O. A. C.—method[15]) with 0.2 *N* $Ba(OH)_2$.

Volatile basic N— 20 cc of the solution [A] was neutralized with 0.2 *N* $Ba(OH)_2$, 0.5 g of MgO and a few drops of octyl alcohol added. and the mixture was put through the aeration method as in a).

Trimethyl amine N— There are many worker's reports on the determination of trmethyl amine, but complicated and slow method, even if it is accurate, cannot be used in follwing the deterioration of freshness. Determination in the present experiment was carried out according to the author's modification of Lintzel's method[14] used for humann urine. This method is based on the fact that ammonia combines with formaldehyd but trimethyl amine does not and is driven out by aeration. Beatty and Gibbons[8] reported a determination method based on this principle for the spoilage in fish.

To 500 cc of the solution [A] was added 15% NaOH solution to render it weakly acid to litmus paper. The solution obtained was evaporated to 40—50 cc volume on a steam bath. To this were added 15 cc of 35% formalin, 15% NaOH until slightly acid to litmus paper, and then, 2 g of MgO and a few drops of octyl alcohol. The mixture was then immediately submitted to aeration, continuing for 4 hrs. at 40°C, into 5 cc of 0.02 *N* H_2SO_4. Excess acid was titrated with 0.02 *N* NaOH with methyl red-methylene blue indicator.

Trimethyl amine-oxide N— The determination was based on the Hoppe-Seyler's method, but aeration was substituted for distillation in the said method.

200 cc of the solution [A] was heated for 1 hr. on an asbestos-wire-netting with 10 cc of hydrochloric acid, and was rendered weakly acidic with NaOH solution. The solution obtained was evaporated on a steam bath to 20—25 cc. 0.5 g of MgO was then added, and by means of a rapid current of air through the mixture at 40°C for 4 hrs., volatile basic substance was driven out. The remainder was centrifuged, and the supernatant clear solution was seperated into a beaker. The precipitate was washed 3 times by centrifuge with successive 5 cc of distillred water. To the all combined supernatant clear solution in the beaker were added hydrochloric acid until slightly acid to congo red paper and then 10 cc of 40% $SnCl_2$. The mixture was heated on an asbestos-wire-netting for 20 min., and then neutralized with NaOH solution. After the addition of 0.5 g MgO to the mixture, trimthyl amine derived from the oxide by reduction was driven out into 0.02 *N* H_2SO_4 by aeration at 40°C for 4 hrs. Excess

acid was titrated with 0.02 *N* NaOH.

From the above experiments, the following results were obtained.

Table V.

Time (hrs)	Volatile basic N*	Formol titrating N*	Trimethyl amine N*	Trimethyl amineoxide N*	Indications
0	20.3	90.6	0.34	1.33	Dark tinge. Faint sour odor.
20	22.8	111	0.35		Distinct sour odor.
44	29.7	136	0.46	1.16	Strong sour odor. Gas generation in the interior.
68	34.9	163			
92	41.8	183	0.57		
140	50.2	216	0.70	1.34	
188	52.9	238			Putrid smell.
236	58.9	260	0.84	1.30	

(* in mg/100 g)

II. Comparison between percentage value of freshness of whale meat at the time of dissection and the amount of volatile basic and formol titrating nitrogen.

Material and tratment :— 18 pieces of meat taken from 9 sperm whales which were caught off the coast of Kinka-zan in August and Ocotver, 1947 were used.

The material was taken from the animal being dissected, and the percentage value of freshness of the material was determined by a practised operator immediately. Without delay, the material was minced and thoroughly mixed. As soon as possible, 4 g of the minced material was weighed into a test-tubs, and 8 cc of absolute alcoholic 0.01 *N* H_2SO_4 was added as a preventive for subsequent decay. Samples thus obtained were taken to laboratory, and the amount of nitrogen determined.

Method of determination :—

Volatile basic N— The sample was thoroughly pounded in a mortar, transferred to the aeration apparatus, using 15 cc of distilled water, and the aeration performed as in a).

Formol titrating N— 40 cc of distilled water was added to the sample in a beaker, and the mixture was heated in a gently boilling water bath for 15 min. under occasional stirring. The liquid was decanted throgh a filter into an Erlenmeyer's flask, and the residue in the beaker washed 3 times with succesive 20 cc portions of distilled water with heating in a water bath for 5 min, each. 2 cc of 20% sulfosalicylic acid was added to

the combined filtrate and washings in the flask. The mixture was allowed to stand overnight, and filtered into 100 cc volumetric flask. The residue on the filter was washed until the volume of combined filtrate and washings reached the marking. Formol titration was performed using 40 cc of the solution obtained.

Results obtained ware as follows:—

Table VI.

Freshness %	Volatile basic N*	Formol titrating N*
90	3.5	61.5
	4.1	69.5
	2.2	62.8
	8.1	71.1
	7.7	79.5
average	5.5	70.7
80	10.6	87.4
	6.2	79.1
	7.6	79.1
	8.6	72.8
	7.9	87.4
average	8.2	81.2
75	10.2	98.8
	9.9	82.2
	8.5	77.8
	17.2	102.1
average	11.5	90.2
70	16.0	—
60	38.6	—
50	246	—
40	453	—

(* in mg/100 g)

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